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From: Leena H. Karttunen	Date: March 9, 2005	No. of Pages: (including this page)	

Comments:

Dear Mr. Cheu:

Further to your phone call today, please find attached Exhibit A which should have accompanied the Amendment.

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Exhibit A

Date 3/9/2005

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Updated information available at this time.

LC-MS analyses of patient synovial fluid for the presence of chlorinated peptide(s). To determine the appropriate parameters for detecting chlorine-containing peptides in the synovial fluid from patients with degenerative joint diseases (DJD), the system was initially set up using a Cl-VIP standard, which is shown in figure 1. The synovial fluids from patients with early or advanced OA were then analyzed and compared with synovial fluid taken from a patient with an acute cruciate ligament (ACL) tear. In brief, an enzymatic digest of the synovial fluid samples was fractionated on a reverse-phase C_{18} column using a 5% acetonitrile to 70% acetonitrile in 0.1% trifluoroacetic acid gradient over a period of 40 minutes. As expected, a number of protein digest products were detected by LC fractionation (UV absorbance 214 nm). Those LC fractions containing protein were further analyzed by positive ion Mass spectrometry. The Mass spectrum peaks were then scanned for the presence of chlorinated peptides using the Micromass software search (MassLynx). The patient samples were also analyzed for the presence of myeloperoxidase (MPO), the enzyme that is responsible for generating the chlorinated products (Bioxytech MPO-Enzyme Immunoassay, OxisResearch, Portland, OR).

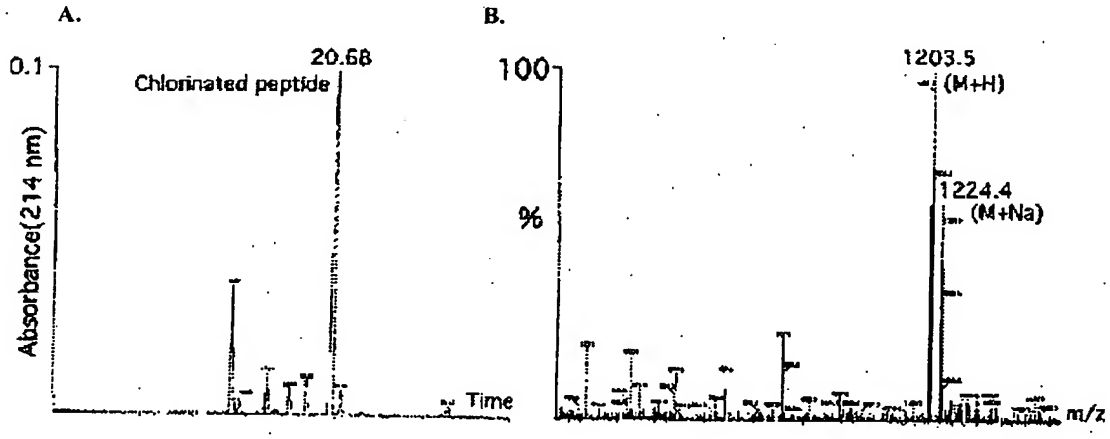

Figure 1. Tryptic digest of the Cl-VIP standard (25 µg) fractionated on a reverse-phase C_{18} column using a 5% to 70% acetonitrile in 0.1% trifluoroacetic acid gradient over a period of 40 minutes and analyzed by MS for the presence of Cl^-. (A) LC reverse phase separation profile of a tryptic digest of Cl-VIP peptide monitored at 214 nm and (B) positive ion-mass spectrum of the 20.68 min LC fraction (5-6 µg) of the N-peptide of Cl-VIP. The mass spectrum of the 20.68 min peak showed a strong (M+H) pseudomolecular ion at m/z 1203.5, with an isotope pattern consistent with the presence of Cl.

Neither the controls nor the advanced OA patient samples had detectable Cl-peptides (Table I). Whereas, 2 patients diagnosed with early OA were positive for the presence of a Cl-peptide (Table I). A Mass spectrum of these peptides demonstrated a strong (M+H) pseudomolecular ion, with an isotope pattern consistent with the presence of Cl. Further characterization of the Cl-peptide is currently underway. These patients also had elevated MPO levels, which is consistent with the presence of a Cl-peptide. The elevated MPO activity in one of the late OA samples was most likely due to the amount of blood present. However, the results indicate that MPO alone is not an adequate biomarker for early DJD.

The preliminary data presented in Table I adds further support to our hypothesis that the presence of Cl-peptides in synovial fluid taken from patients with DJD can be used as a biomarker for the early diagnosis of these disease processes.

Table I. Summarizes the MPO and Cl-peptide results for synovial fluid samples, and the relevant patient information that may contribute to the onset or severity of OA.

Synovial Fluid Sample #	Fluid volume/	Diagnosis	Race/ sex	Height/Weight	Age	Cl-peptides /Molecular Mass (Da)	MPO ng/ml
001	10 ml	Torn ACL	W/m	72"/185 lbs	36	Negative	0
023	0.1 ml	Torn Meniscus	W/m	70"/200 lbs	38	Negative	0
002	1 ml	Early OA	B/f	62"/282 lbs	31	Positive/ 808.3 Da	52
015	6.0 ml	Early OA	B/f	62"/200 lbs	72	Negative	4
017	5.5 ml	Early OA	B/f	62"/222 lbs	52	Positive 1908.9 Da	74
003	2 ml	Advanced OA	W/f	76"/145 lbs	63	Negative	4.5
004	3 ml Bloody tap with WBCs	Advanced OA	W/f	68"/200 lbs	76	Negative	40 (High WBC count)
024	7.5 ml	Advanced OA	W/f	62"/209 lbs	61	Negative	5
025	6.5 ml	Advanced OA	W/m	72"/244 lbs	74	Negative	32
027	2.5 ml	Advanced OA	W/f	62"/236 lbs	59	Negative	7